Interaction of *Fusarium moniliforme* and *Aspergillus flavus* on Kernel Infection and Aflatoxin Contamination in Maize Ears

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ABSTRACT

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Fusarium moniliforme is frequently recovered from symptomless maize kernels from ears inoculated in the field with *Aspergillus flavus* in Mississippi. When maize ears were inoculated simultaneously with *F. moniliforme* and *A. flavus* or with *A. flavus* alone in 1990, significantly fewer kernels were infected with *A. flavus* in ears inoculated with both fungi than kernels from ears inoculated with *A. flavus* alone. Grain from ears inoculated with both fungi had significantly less aflatoxin than grain from ears inoculated with *A. flavus* alone in two tests in 1990. Inoculation of ears with *A. flavus* alone in 1989 resulted in significantly more natural infection of kernels by *F. moniliforme*. In contrast, percentages of natural infection of kernels by *A. flavus* in ears inoculated with *F. moniliforme* alone and in uninoculated ears were both low and did not differ significantly. Apparently, *F. moniliforme* can inhibit kernel infection by *A. flavus* in inoculated maize ears and lead to reduced aflatoxin contamination in these kernels.

Fusarium moniliforme Sheld., one of the most cosmopolitan of plant pathogens, is found in most soils where maize (*Zea mays* L.) can be grown. The fungus persists on plant residues and organic matter in or on the soil and may invade maize plants whenever the environment becomes favorable. It may cause seedling blight, root or stalk rot, pokkah boeng, and kernel or ear rot. Koehler (5) reported that *F. moniliforme*, the incitant of *Fusarium* ear rot, enters the ear through the silk channel, spreads within the ear on the silks, and infects isolated single kernels or groups of kernels in localized areas of the ear. Growth cracks in the pericarp or other damage enhances the infection of kernels by the pathogen, but the fungus can penetrate the pedicels of intact kernels.

King and Scott (4) reported high levels of asymptomatic kernel infection by *F. moniliforme* in commercially grown maize in Mississippi. King (3) found that *F. moniliforme* could be isolated from maize kernels 2 wk after midsilk and determined that infection increased weekly to 35-66% throughout the season. Scott and King (8) reported that the genotype of the pericarp in maize conditions resistance to kernel infection by *F. moniliforme*.

Zummo and Scott (12) frequently isolated *F. moniliforme* from symptomless cobs and kernels from ears that were inoculated with *Aspergillus flavus* Link ex Fries, as well as from cobs and kernels from uninoculated ears. They found that significantly more pedicel than apical portions of kernels were infected with *F. moniliforme*. In contrast, *A. flavus* was detected more frequently in apical and middle portions of kernels than from pedicel portions. They concluded that *A. flavus* penetrates maize kernels mainly through the pericarp.

Wicklow et al (10) investigated the ability of "competing fungi" in preharvest maize to interfere with *A. flavus* infection and aflatoxin contamination of developing kernels. They concluded that the competing fungi, particularly *F. moniliforme*, could inhibit kernel infection by *A. flavus* and aflatoxin contamination of other uninjured kernels on the same ear. Hill et al (2) reported that positive and negative correlations occurred in 1978 and 1979 between members of the *A. flavus* group and other microorganisms on and within maize kernels with the occurrence of aflatoxins and with damage to the kernels. They found that *F. moniliforme* occurred more abundantly than members of the *A. flavus* group. Negative correlations between the *A. flavus* group and *F. moniliforme* occurred consistently both years for individual planting dates with sampling dates of 45 and 60 days after full silk.

The colonization of maize kernels by *A. flavus* and the subsequent production of aflatoxin by the fungus poses a serious economic problem to maize production in the southeastern region of the United States (1,7). Maize grain containing violative amounts of aflatoxin cannot be sold in interstate commerce. Maize genotypes resistant to kernel infection have been identified (9). Because both fungi can enter maize kernels through the pericarp, and earlier work indicated that *F. moniliforme* could interfere with the infection process of certain fungi, identification of sources of resistance may be influenced by the presence of *F. moniliforme* in maize ears. We undertook these studies to demonstrate the effect of *F. moniliforme* on kernel infection and aflatoxin production by *A. flavus* in inoculated maize ears.

MATERIALS AND METHODS

Field plots, harvest, and handling methods. The maize assayed in this study was grown in replicated single-row plots at the Plant Science Center, Mississippi State, MS. Each single-row plot was 5 m long and spaced 1 m from adjacent plots. Each plot was overseeded and thinned to 20 plants spaced approximately 25 cm apart. The top ear of each plant was harvested 60 days after midsilk, at which time the moisture content of the kernels was approximately 14-17%. Immediately after harvest, the ears were dried at 42 C for 7 days in a forced air dryer to a kernel moisture content of approximately 10%, then shelled mechanically. Kernels from each plot were bulked in paper bags and stored at 6 C and 45% relative humidity until assayed.

Inoculum, inoculation techniques, and assays. *A. flavus* isolate (NRRL 3357) obtained from Stephen W. Peterson, Northern Regional Research Center, Peoria, IL, was used to produce inoculum in each year of these studies. *F. moniliforme* was isolated from naturally infected maize kernels at Starkville, MS, and its identity was verified by Paul Nelson, Pennsylvania State University, State College, PA. Cultures of each fungus were grown on corn cob grits in 500-ml Erlenmeyer flasks, each containing 50 g of grits and 100 ml of H_2O. After 12-14 days, conidia of *A. flavus* and microconidia of *F. moniliforme* were washed from the surface of the grits with sterile distilled water containing two drops of Tween 20 per 100 ml. Microconidia of *F. moniliforme* were used as inoculum because the fungus did not produce sufficient macroconidia for our use. Inoculum was prepared daily and kept on ice in the field until applied.

Ears were inoculated 6 days after midsilk. A tree-marking gun fitted with a 14-gauge hypodermic needle, 35 mm long

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with the tip opening plugged and three 1-mm holes drilled 6, 8, and 10 mm from the tip (11) was inserted through the husks and 3.4 ml of inoculum containing 9×10^6 conidia or microconidia per milliliter was injected over the kernels without visibly damaging them. When ears were inoculated with both fungi simultaneously, the spore concentration of each was doubled and equal quantities of suspension were mixed so that 3.4 ml of inoculum containing the same number of conidia or microconidia for inoculating with a single fungus was used. Ears were shelled, kernels were bulked, and a random sample was selected for assay.

Three hundred ninety undamaged kernels from each plot were plated on Czapek solution agar amended with 7.5% NaCl (CSA-S). The kernels had been dipped momentarily in 70% ethanol, submerged in 1.25% NaOCl for 3 min, and rinsed in sterile, distilled water to eliminate surface microbes. Then the kernels were plated on 100-mm petri dishes (13 kernels per plate). The plates were incubated for 7 days at 28 C and then examined for fungal growth.

The procedure recommended by the Vicam Company, Somerville, MA, was followed to determine aflatoxin concentration in the grain. A 60-g sample of kernels from each plot was ground in a Straub Model 4E grinding mill (Straub C., Philadephia, PA). Fifty grams of finely ground corn and 5 g NaCl were blended with 100 ml of MeOH-H_2O (8:2) for 60 sec. The mixture was filtered through M901 filter paper (Schleicher & Schuell Inc., Keene, NH), and 10 ml of filtrate was mixed with 40 ml of H_2O. This solution was filtered through glass filter paper (ZE 903, Schleicher & Schuell Inc., Keene, NH), and 10 ml was passed through an Aflatest P immunoaffinity column (Vicam, Somerville, MA) with light pressure supplied by a glass syringe and plunger. The affinity column was washed twice with 10 ml of H_2O, and the aflatoxin was eluted with 1 ml of MeOH. The eluent was combined with 1 ml of bromine developer (0.002% Br2), mixed, and the level of fluorescence determined in a fluorometer (TorBex model FX-100 Series-3, Vicam, Somerville, MA). The fluorometer reading was in nanograms per gram of aflatoxin.

Field experiments. In 1988, six maize hybrids (Ring Around 1502, Coker 3020, Cargill 8967, NK PX9581, Sunbelt 5613, and Pioneer Brand 3369A) were compared for percentage of kernel infection when ears were inoculated with 1) *A. flavus* alone, 2) *F. moniliforme* alone, or 3) both *A. flavus* and *F. moniliforme*. Ears in uninoculated plots of these hybrids served as a control. The field design was a randomized complete block with four replications of single-plot rows as described above.

In both 1989 and 1990, four maize hybrids (Mp313E × Mp337, Mp420 × Tx601, GA209 × Mp339, and CI21 × GA203) were compared for percentage of kernel infection and aflatoxin contamination in the grain when ears were inoculated with 1) *A. flavus* alone, 2) *F. moniliforme* alone, 3) *A. flavus* plus *F. moniliforme*, and 4) not inoculated. The field design was a randomized complete block with six replications of single plot rows as described above. The experiment was repeated in a second crop planted 30 days after the first. Planting dates for the first crop were 18 and 17 April in 1989 and 1990, respectively.

Analysis of data. Data were subjected to a standard analysis of variance using the percentage of 390 kernels infected with *A. flavus* and/or *F. moniliforme* or aflatoxin content as a plot mean. The means were separated using LSD. Neither arcsine nor square root of $X + 1$ transformations indicated any significant differences not detected by the analysis of the original percentages.

Table 1. Percentage of kernels infected with *Aspergillus flavus* and *Fusarium moniliforme* in ears of six maize hybrids needle-inoculated with one or both fungi in the field at Mississippi State, MS, in 1988

Inoculation treatment	Hybrids[a] 1	2	3	4	5	6	Treatment means
A. flavus infection (%)							
A. flavus	4.8[b]	6.4	9.9	5.9	15.7	4.8	7.9
F. moniliforme	1.8	0.8	1.5	3.9	1.0	1.1	1.7
A. flavus + *F. moniliforme*	2.6	1.7	3.5	1.6	1.4	2.7	2.2
Uninoculated	4.8	2.1	1.6	1.4	1.8	2.8	2.8
LSD (0.05) for treatment means =							3.8
F. moniliforme (%)							
A. flavus	14.3	35.0	42.0	37.4	28.4	41.0	33.0
F. moniliforme	57.5	81.8	71.8	76.4	68.9	72.5	71.5
A. flavus + *F. moniliforme*	33.4	72.6	71.0	79.9	68.7	74.4	66.7
Uninoculated	16.4	47.6	39.4	45.3	29.8	36.4	35.8
LSD (0.05) among treatment means =							6.1

[a] 1, Ring Around 1502; 2, Coker 3020; 3, Cargill 8967, 4, NK PX9581; 5, Sunbelt 5613; 6, Pioneer Brand 3368A.

[b] Each value is mean percentage of kernels infected with *A. flavus* or *F. moniliforme* in four replications of 390 kernels after surface-sterilization and 7 days of incubation at 28 C on Czapek solution agar amended with 7.5% NaCl.

Table 2. Percentages of kernels infected with *Fusarium moniliforme* and *Aspergillus flavus* in ears of four maize genotypes needle-inoculated with one or both fungi in the field at Starkville, MS, in 1989

Inoculation treatment	*A. flavus* Test 1 (%)	*A. flavus* Test 2 (%)	*F. moniliforme* Test 1 (%)	*F. moniliforme* Test 2 (%)
A. flavus	3.5[a]	4.6	17.5	10.9
F. moniliforme	1.2	2.0	26.1	21.7
A. flavus + *F. moniliforme*	3.2	3.8	33.5	27.3
Check	1.6	1.5	3.1	3.6
LSD (0.05)	1.2	2.0	8.1	8.8

[a] Each value is mean percentage of kernels infected with *A. flavus* or *F. moniliforme* in 24 assays of 390 kernels after surface-sterilization and 7 days of incubation at 28 C on Czapek solution agar amended with 7.5% NaCl.

RESULTS AND DISCUSSION

There were significantly fewer kernels infected by *A. flavus* in plots inoculated with *A. flavus* and *F. moniliforme* than in plots inoculated with *A. flavus* alone in 1988 (Table 1). So, with inoculated ears, infection of kernels by *F. moniliforme* inhibited infection by *A. flavus*. The significant hybrid × fungus treatment interaction for *A. flavus* reflected differences in magnitude of hybrid response rather than opposite reactions. The high infection value for *A. flavus* in hybrid 5 contributed most to the hybrid × treatment interaction (Table 1). The hybrid × treatment interaction means square was used to calculate the LSD for treatment means. Kernel infection by *F. moniliforme* did not differ between the treatments of *F. moniliforme* alone and *A. flavus* + *F. moniliforme*, indicating that *A. flavus* did not inhibit kernel infection by *F. moniliforme*.

In both tests in 1989, differences in kernel infection by *A. flavus* between *A. flavus* and *A. flavus* × *F. moniliforme* treatments were not significant (Table 2). Also, there was no indication that *A. flavus* inhibited kernel infection by *F. moniliforme*. There was evidence that inoculation with *A. flavus* alone enhanced kernel infection by *F. moniliforme*. The interpretation or importance of this finding is not readily apparent.

Kernel infection by *A. flavus* was significantly less in both tests inoculated with *A. flavus* and *F. moniliforme* than in plots inoculated with *A. flavus* alone in 1990 (Table 3). There was no hybrid

Table 3. Percentages of kernels infected with *Fusarium moniliforme* and *Aspergillus flavus* in ears of four maize genotypes needle-inoculated with one or both fungi in the field at Starkville, MS, in 1990

	A. flavus		*F. moniliforme*	
Inoculation treatment	**Test 1 (%)**	**Test 2 (%)**	**Test 1 (%)**	**Test 2 (%)**
A. flavus	4.7[a]	6.0	10.7	14.9
F. moniliforme	1.9	1.6	26.5	21.2
A. flavus + *F. moniliforme*	2.1	3.3	19.3	22.5
Check	1.6	2.1	5.9	9.9
LSD (0.05)	1.0	1.6	5.3	5.0

[a]Each value is mean percentage of kernels infected with *A. flavus* or *F. moniliforme* in 24 assays of 390 kernels after surface-sterilization and 7 days of incubation at 28 C on Czapek solution agar amended with 7.5% NaCl.

Table 4. Aflatoxin concentrations in kernels of ears of four maize hybrids inoculated in the field with *Aspergillus flavus* and/or *Fusarium moniliforme* at Starkville, MS, in 1990

	Aflatoxin concentration	
Inoculation treatment	**Test 1 (ng/g)[a]**	**Test 2 (ng/g)**
A. flavus	42.5	51.4
F. moniliforme	5.3	1.1
A. flavus + *F. moniliforme*	24.1	19.4
Check	1.6	1.0
LSD (0.05)	16.0	14.7

[a]Each value is the average fluorometer reading from plots of six replications of four maize genotypes.

× treatment interaction for kernel infection by *A. flavus* in 1990. In test 1, kernel infection by *F. moniliforme* was higher in the treatment for *F. moniliforme* alone than when inoculated with both fungi indicating that *A. flavus* inhibited infection by *F. moniliforme*. However, this was not found in test 2. As in 1989, there was a suggestion that inoculation with *A. flavus* enhanced the infection rate by *F. moniliforme*.

There was significantly less aflatoxin in kernels from plots inoculated with both *A. flavus* and *F. moniliforme* than in plots inoculated with *A. flavus* alone in both aflatoxin tests in 1990 (Table 4). A significant hybrid × treatment interaction occurred only in the second test, but significant treatment effects were present in both tests. The significant interaction of test 2 resulted because two hybrids had significantly less aflatoxin in plots inoculated with *A. flavus* and *F. moniliforme* than in plots inoculated with *A. flavus* alone, but the other two hybrids did not exhibit this reduction.

We did not determine what part of the reduction in aflatoxin accumulation in kernels from ears inoculated with both fungi was due to reduction in infection by *A. flavus* or what part was due to reduced aflatoxin production by the fungus. The results obtained in our tests are in line with those of Wicklow et al (10) and Hill et al (2).

Kernel infection by *A. flavus* was found here and suggested earlier (2,10) to be inhibited by infection with *F. moniliforme*. Thus, high populations of *F. moniliforme* may interfere with the detection of resistance to *A. flavus* in field-grown corn, particularly where breeders rely on natural inoculation by *A. flavus*. The level of interference might be affected by the time of inoculation or infection. As noted above, King and Scott (3) isolated *F. moniliforme* from kernels 2 wk after midsilk but not earlier, whereas Marsh and Payne (6) found that *A. flavus* infected ears during silking. Therefore, under natural conditions, *A. flavus* may infect ears before *F. moniliforme*. Unfortunately, with field-grown corn, the dispersal and size of populations of *A. flavus* cannot be controlled such that developing silks are reliably inoculated with large populations of this fungus. In contrast, with artificial inoculation of ears, the concentration and timing of the inoculation are controlled. The number of propagules of *A. flavus* introduced into potential infection courts probably precludes infection by populations of *F. moniliforme* that occur naturally because the latter are unlikely to be directly introduced into the same infection courts. So, with the artificial inoculation of ears by *A. flavus*, the presence of *F. moniliforme* is not likely to confound the detection of resistance to infection by *A. flavus* or the accumulation of aflatoxin.

Results obtained in this study show that *F. moniliforme* does not prevent identification of genotypes resistant to *A. flavus*, because the selected resistant hybrid, Mp313E × Mp337, averaged 3.2% kernels infected by *A. flavus* over the 2 yr of tests compared to 6.1% for a susceptible hybrid, C121 × GA202. However, if these hybrids could be tested in a an environment free of *F. moniliforme*, differences might be greater and, thus, easier to detect.

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